REMARKS

Annex A to form PCT/RO/106 indicates that the International Application is defective in not meeting the requirements of PCT Rules 4.15 and 90.4 inasmuch as the application was signed by an agent/common representative but was not accompanied by a power of attorney appointing the agent/common representative. Accordingly, powers of attorney (signed by Applicant for all designated states except the U.S., and by the Inventors for the U.S. only) are submitted herewith.

Annex B1 to Form PCT/RO/106 indicates pages 92-93 and 105-106 of the application are defective in not meeting the requirements of PCT Rules 11 and 26.3(a)(i) because the right margin of these pages is not 2cm. Substitute pages 92-93 and 105-106, in which the right margin is 2cm, are submitted herewith. In addition, typographical errors on pages 3-6, 8, 11-12, 20-21, 37-38, 52, 68, 71-72, 81, 86, 92-93, 98, 100-101, 103, 105-108, 110, 111-112, 133, 144-145, and 148 have been corrected as described above, and corresponding substitute pages are submitted herewith. These typographical errors are obvious and the correction thereof does not involve new matter.

Annex C1 to form PTO/RO/106 indicates that the drawings of the International Application as filed do not meet the physical requirements specified by PCT Rules 11 and 26.3(a)(i) because: the left margin of Figure 2 is not the prescribed minimum (2.5cm); the sheets are not numbered in consecutive Arabic numerals; and Figures 1 and 2 contain numbers, letters and reference lines lacking simplicity and clarity. Formal drawings meeting the physical requirements prescribed by PCT Rules 11 and 26.3(a) are submitted herewith.

CONCLUSION

In view of the foregoing, the International Application now fulfills the requirements of PCT Rules 4.15, 11, 26.3(a), 26.4(a), and 90.4.

Respectfully submitted,

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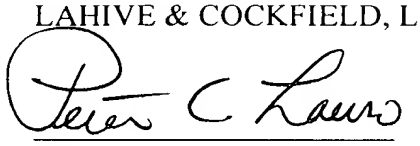

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Attachments: (Powers of Attorney; Formal Drawings; Substitute specification pages 3-6, 8, 11-12, 20-21, 37-38, 52, 68, 71-72, 81, 86, 92-93, 98, 100-101, 103, 105-108, 110, 111-112, 133, 144-145, and 148.)

assayed for their ability to complement a gpal null phenotype (i.e., constitutive activation of the pheromone response pathway) in S. cerevisiae, a situation in which it was desirable to retain a substantial portion of the GPA sequence. Clearly, a method for optimizing the functional integration of a heterologous GPCR into a signaling pathway in a yeast cell expressing such a receptor would be of great value in developing assays to identify receptor agonists and antagonists.

Summary of the Invention

The present invention provides an important advance in drug screening methodologies previously known in the art by providing, *inter alia*, a means by which expression of heterologously expressed receptors is enhanced and a means by which coupling of heterologously expressed GPCRs to G protein subunits is enhanced.

The present invention pertains to novel yeast cells which are useful for the expression of functional heterologous GPCRs. In certain embodiments, the subject yeast cells comprise modified and/or heterologous G protein subunits which enhance the functional integration of heterologous GPCRs into a yeast signaling pathway. The modified G protein subunits can be altered by mutation and/or can be chimeric, i.e., can comprise a polypeptide derived from a yeast G protein subunit and one or more polypeptides derived from heterologous G protein subunits. The yeast cells of the present invention can be used in novel screening assays which can be used to screen for modulators of GPCRs.

In one aspect, the invention is a recombinant yeast cell which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell; and

a chimeric G protein subunit selected from the group consisting of:

a non-naturally occurring G protein subunit which comprises a sequence from a heterologous G protein subunit in which at least one amino acid substitution has been introduced compared to the wild type sequence;

an endogenous STE 18 subunit operably linked to a polypeptide of a heterologous Gγ subunit;

an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit; and

an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which the N-terminus of said Gpa1 is operably linked to at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different; such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone system pathway of said yeast cell; and

wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

In one aspect, the invention provides a yeast cell comprising a heterologous G protein coupled receptor; and a non-naturally occurring G protein subunit which has a sequence from a heterologous G protein subunit, but in which at least one amino acid substitution has been introduced compared to the wild type sequence. The expression of the non-naturally occurring G protein subunit functionally integrates the heterologous G protein coupled receptor into the yeast cell pheromone signaling pathway.

In one embodiment, the yeast cell of the present invention has a non-naturally occurring G protein subunit which is a mutant mammalian Gα subunit. In preferred embodiments, the mutant mammalian Gα subunit comprises a sequence from a heterologous G protein subunit having a mutation selected from the group consisting of: Gα16(S270P); Gαs(D229S); Gαs(D229V); Gαs(N254D); Gαs(S286P); Gαs (E10K); Gαi2-GαoB (S280P); Gα12 (Q229L); Gα12 (G228A); and Gαi2 (S288P).

In another embodiment, the yeast cell of the present invention comprises a non-naturally occurring G protein subunit which is a yeast-mammalian G protein subunit chimera comprising a first polypeptide from a yeast G protein subunit and a second polypeptide from a mutant mammalian G protein subunit. In preferred embodiments the second polypeptide of the chimera comprises a mutant mammalian Gα subunit selected from the group consisting of: Gα16(S270P); Gαs(D229S); Gα s(D229V); Gαs(N254D); Gαs(S286P); Gαs (E10K); Gαi2-GαoB (S280P); Gα12 (Q229L); Gα12 (G228A); and Gαi2 (S288P).

In another preferred embodiment, a yeast cell of the present invention has a chimeric G protein subunit, which comprises a first polypeptide from a yeast G protein subunit and a second polypeptide from a heterologous G protein subunit, where the first polypeptide is selected from the group consisting of: a polypeptide comprising about 40

amino acids from the amino terminus of yeast GPA1; and a polypeptide from yeast STE 18.

In yet another preferred embodiment, a yeast cell of the present invention has a chimeric G protein subunit where the first polypeptide of the chimera comprises about 40 amino acids from the amino terminus of yeast GPA1 and said second polypeptide of the chimera is from a heterologous G protein α subunit.

In yet another embodiment a yeast cell of the present invention has a chimeric G protein subunit in which the first polypeptide is from yeast STE 18 and the second polypeptide is from a heterologous G protein γ subunit.

In preferred embodiments, a heterologous G protein subunit of the present invention is mammalian. In particularly preferred embodiments, a heterologous G protein subunit of the present invention is human.

In one embodiment, a yeast cell of the present invention comprises a chimeric G protein subunit in which at least one of the first and second polypeptides comprises a naturally occurring amino acid sequence. In still another embodiment, at least one of the first and second polypeptides of the chimeric G protein subunit comprises a non-naturally occurring amino acid sequence.

In preferred embodiments, a yeast cell of the present invention comprises a heterologous G protein coupled receptor which is functionally integrated into the yeast cell.

In particularly preferred embodiments, a modified or chimeric G protein subunit of the present invention demonstrates enhanced coupling to a heterologous G protein coupled receptor expressed by a yeast cell when compared to that demonstrated by an endogenous yeast G protein subunit.

In one embodiment a yeast cell of the present invention comprises a chimeric G protein subunit in which the second polypeptide is from the human Gγ2 subunit. In a preferred embodiment, the second polypeptide comprises the amino acid sequence Arg Glu Lys Lys Phe Phe (amino acids 19-24 of SEQ ID NO: 33). In a particularly preferred embodiment, the chimeric G protein subunit comprises the sequence shown in SEQ ID NO: 33.

In a preferred embodiment, a yeast cell of the present invention comprises a chimeric G protein subunit selected from the group consisting of: gpal (41)-Gαi2; gpal (41)-Gα16; and gpal (41)-Gαs. In a more preferred embodiment, a yeast cell of the present invention comprises a chimeric G protein subunit in which the Gαi2, Gα16, or Gαs portion of the chimeric G protein subunit comprises an amino acid substitution compared to wild type Gαi2, Gα16, or Gαs.

In yet another embodiment, a yeast cell of the present invention comprises a second chimeric G protein subunit, in which the second chimeric G protein subunit has a first polypeptide from a yeast G protein subunit and a second polypeptide from a mammalian G protein subunit, and wherein the second chimeric G protein subunit is different from a first chimeric G protein subunit expressed by the yeast cell. In a preferred embodiment, the second polypeptide of the second chimeric G protein subunit is from a protein selected from the group consisting of: a mammalian Gα subunit, a mammalian Gβ subunit, and a mammalian Gγ subunit.

In preferred embodiments, a yeast cell of the present invention does not produce an endogenous yeast pheromone system receptor protein in functional form.

In certain embodiments, a yeast cell of the present invention comprises a indicator gene that produces a detectable signal upon functional coupling of the heterologous G protein coupled receptor to the G protein.

In preferred embodiments, a yeast cell of the present invention comprises a heterologous G protein coupled receptor which is an orphan receptor.

In another embodiment the invention provides an assay to identify compounds capable of modulating the dissociation of Gα and Gβγ, comprising the steps of: providing a yeast cell which comprises a heterologous G protein coupled receptor, a modified G protein subunit, and an indicator gene, contacting the yeast with a test compound; and identifying compounds which induce a change in a detectable signal in the yeast cell, wherein said detectable signal indicates dissociation of Gα and Gβγ.

In certain embodiments, an assay of the present invention is used to test compounds from a library of non-peptidic organic molecules.

In another aspect, the invention provides a method for identifying a compound which modulates a heterologous G protein coupled receptor, comprising: providing a first, second, third, and fourth yeast cell, each cell comprising a G protein, wherein:

1) the first yeast cell comprises a first chimeric G protein subunit comprising a first polypeptide from a yeast G protein subunit and a second polypeptide from a mammalian G protein subunit; 2) the second yeast cell comprises a second chimeric G protein subunit comprising a first polypeptide derived from a yeast G protein subunit and a second polypeptide from a mammalian G protein subunit, the second chimeric G protein subunit being different from said first chimeric G protein subunit; 3) the third yeast cell comprises a third chimeric G protein subunit comprising a first polypeptide from a yeast G protein subunit and a second polypeptide from a mammalian G protein subunit, the third chimeric G protein subunit being different from said first

In one embodiment of the invention, the heterologous G protein coupled receptor which is expressed by a yeast cell is an orphan receptor.

In still yet another aspect, the invention is directed to carboxy terminal chimeric G protein subunits, and sandwich chimeric G protein subunits. Thus, in one embodiment, a yeast cell of the invention includes a chimeric G protein subunit comprising an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit.

In another embodiment, a yeast cell of the invention includes a chimeric G protein subunit comprises an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, and in which the N-terminus of said Gpa1 is operably linked to at least the first five N-terminal amino acids of a heterologous G protein subunit, wherein said heterologous G protein subunits are the same or different.

In another embodiment, a yeast cell of the invention which comprises:

a heterologous G protein-coupled receptor (GPCR) expressed in the cell membrane of said yeast cell such that signal transduction activity via said receptor is modulated by interaction of an extracellular region of the receptor with an extracellular signal, said heterologous GPCR acting as a surrogate for an endogenous yeast pheromone receptor in a pheromone response pathway of the yeast cell; and

a chimeric G protein subunit comprising an endogenous yeast Gpa1 subunit in which at least the last four C-terminal amino acids are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit, such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

In another embodiment, the yeast cell comprises a heterologous polypeptide, wherein the heterologous polypeptide is transported to a location allowing interaction with the extracellular region of the receptor expressed in the cell membrane; and wherein the heterologous polypeptide is expressed at a sufficient level such that modulation of the signal transduction activity of the receptor by the heterologous polypeptide provides a detectable signal.

In yet another embodiment, the yeast cell comprises a reporter construct that is activated by the pheromone response pathway, wherein the heterologous

protein subunit, wherein said first and second heterologous G protein subunits are the same or different; such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and

wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

In a preferred embodiment, the chimeric G protein subunit comprises an endogenous yeast Gpa1 subunit in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a first heterologous G protein subunit, and in which the first five N-terminal amino acids of said Gpa1 are replaced with the first 11 N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same.

In another preferred embodiment, the chimeric G protein subunit comprises an endogenous yeast Gpa1 subunit in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a first heterologous G protein subunit, and in which the first 21 N-terminal amino acids of said Gpa1 are replaced with the first 21 N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same.

In another aspect, the invention is a chimeric G-protein subunit which comprises an endogenous Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit.

In a preferred embodiment, the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a heterologous G protein subunit.

In another preferred embodiment, the last six C-terminal amino acids of said Gpa1 are replaced with the last six C-terminal amino acids of a heterologous G protein subunit.

In another embodiment, the invention is a chimeric G-protein subunit which comprises an endogenous Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which the N-terminus of said Gpa1 is operably linked to at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different.

In a preferred embodiment, the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of said first heterologous G-protein subunit, and in which the first five N-terminal amino acids of said Gpa1 are replaced with the first 11 N-terminal amino acids of said second heterologous G protein subunit.

In another preferred embodiment, the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of said first heterologous G-protein subunit, and in which the first 21 N-terminal amino acids of said Gpa1 are replaced with the first 21 N-terminal amino acids of said second heterologous G protein subunit.

In another preferred embodiment, the first and second heterologous G protein subunits are the same.

In another aspect, the invention is a method for identifying a modulator of a heterologous G protein-coupled receptor expressed by a yeast cell, comprising:

contacting a mixture of yeast cells of the invention with a test compound;

allowing cells within the mixture to generate a detectable signal; and

identifying the test compound as a modulator of said receptor.

In another embodiment, the invention is method for identifying a modulator of a heterologous G protein-coupled receptor expressed by a yeast cell, comprising:

contacting a mixture of yeast cells of the invention with a ligand of said receptor;

allowing cells within the mixture to generate a detectable signal; and

identifying a heterologous polypeptide expressed by the yeast cells as a modulator of said receptor.

In another embodiment, the invention is a method for identifying a modulator of a heterologous G protein-coupled receptor expressed by a yeast cell, comprising:

contacting a first mixture of yeast cells as claimed in claim 78 with a second mixture of yeast cells, wherein collectively the second mixture of yeast cells expresses a library of heterologous test polypeptides that are transported to a location allowing interaction with the extracellular region of said receptor expressed in the cell membrane of the yeast cells of the first mixture;

which will not activate the receptor which is expressed. In a library of such cells, in which a multitude of different peptides are produced, it is likely that one or more of the cells will be "autocrine" in the stricter sense of the term.

As used herein the term "chimeric" G protein subunit refers to a G protein subunit composed of at least two discrete polypeptides, a first polypeptide from a yeast G protein subunit and a second polypeptide from a heterologous G protein subunit. Each of the first and second polypeptides are encoded by a nucleic acid construct and are operatively linked such that upon expression of the construct, a functional chimeric G protein subunit is produced, i.e., a fusion protein comprising the first polypeptide linked to the second polypeptide. In preferred embodiment, the heterologous G protein subunit is mammalian. In particularly preferred embodiments, the heterologous G protein subunit is human. For example, chimeric G protein subunits of the present invention can comprise a polypeptide from GPA1 linked to Gα, STE18, linked to Gγ, or STE4, linked to Gβ. In preferred embodiments, in particular for chimeric Gα subunits, the portion of the chimeric subunit from yeast GPA1 comprises a portion of the amino terminus of GPA1 and is less than 330 amino acids in length. In particularly preferred embodiments, the portion of the chimeric subunit derived from GPA1 is about 40 amino acids. In another embodiment, the portion of the chimeric subunit derived from GPA1 is about 20 amino acids.

As used herein, the term "not produced in functional form" with regard to endogenous yeast proteins is intended to encompass proteins which are not produced in functional form for any number of reasons, for example, because of a mutation to the gene which encodes the protein or a deletion, e.g., a disruption, of the gene which encodes the protein. The term "not produced in functional form" is also intended to include conditional mutations (e.g. temperature sensitive mutation, wherein the protein is not produced in functional form under certain conditions.

As used herein, the terms used to indicate amino acid mutations, such as "S270P" and the like, represent the wild type amino acid residue (in standard one letter code), followed the amino acid position, followed by the substituted amino acid (in standard one letter code). Thus, S270P indicates substitution of the wild type serine at position 270 with proline. The terms such as "Gαs(S270P)" and the like represent the G protein having the indicated substitution. Thus, the term Gαs(S270P) represents a Gαs subunit having a proline substituted for the wild type serine at position 270.

The phrase "last e.g. four C-terminal amino acids" and "first, e.g., 6 N-terminal amino acids" as used herein with reference to polypeptides wherein the amino acids of the polypeptides are read in sequential order from left to right, with the N-

terminus of the polypeptide being at the far left and the C-terminus being at the far right. For example, in a polypeptide of 50 amino acids, the "last five C-terminal amino acids" would refer to amino acids 46 through 50. Similarly, the "first six N-terminal amino acids" would refer to amino acids 1 though 6.

The term "transport to a location", as used herein refers to a heterologous polypeptide which is produced in one region of the yeast cell but which moves to another region of the yeast cell.

The term "expressed at a sufficient level", as used herein refers to a heterologous polypeptide which is produced in an amount capable of modulating signal transduction activity of a receptor.

The term "first heterologous G protein subunit" is used interchangeably with the term "first heterologous G α subunit", and refers to a member of the family of G α subunits that is used to replace the C-terminal or N-terminal amino acids of the Gpa1 protein. The "first heterologous G protein subunit" may also be operably linked to the N-terminal of the Gpa1 protein. Examples of G α subunits include, Gα16; Gαs; Gα s; Gαs; Gαs; Gαs ; Gαi2; GαoB; Gα12; Gα12 ; and Gαi2. The term "second heterologous G protein subunit" is used interchangeably with the term "second heterologous G α subunit", and refers to a member of the family of G α subunits that is used to replace the C-terminal or N-terminal amino acids of the Gpa1 protein. In chimeric G proteins, the Gpa1 protein can be replaced with a first heterologous G protein subunit at the C-terminal of the Gpa1 protein. In "sandwich chimera G proteins", the C-terminal amino acid of the Gpa1 protein can be replaced with a first heterologous G protein subunit, and the N-terminal can be replaced with, or operably linked to, a second heterologous G protein subunit. The first and second heterologous G protein subunits can be the same, for example, Gq-Gpa1-Gq, Gs-Gpa1-Gs and the like, or the first and second heterologous G protein subunits can be different, for example Gq-Gpa1-Gs, Gα-Gpa1-Gq, and the like.

With regard to polypeptides, the terms "operatively linked" and "operably linked", are used herein interchangeably and are intended to mean that two polypeptides are connected in manner such that each polypeptide can serve its intended function. Typically, the two polypeptides are covalently attached through peptide bonds. The fusion protein is preferably produced by standard recombinant DNA techniques. For example, a DNA molecule encoding the first polypeptide is ligated to another DNA molecule encoding the second polypeptide, and the resultant hybrid DNA molecule is expressed in a host cell to produce the fusion protein. The DNA molecules are ligated to each other in a 5' to 3' orientation such that, after ligation, the translational frame of the

Additional sequence information is provided by Mattera, *et al.* (1986, FEBS Lett 206:36-41), Bray, *et al.* (1986, Proc. Natl. Acad. Sci USA 83:8893-8897) and Bray, *et al.* (1987, Proc Natl. Acad Sci USA 84:5115-5119).

As indicated above, there is little if any sequence homology shared among the amino termini of Gα subunits. The amino terminal domains of Gα subunits that precede the first β-sheet (containing the sequence motif -LLLLGAGESG-(SEQ ID NO:81); see Noel, *et al.* (supra) for the numbering of the structural elements of Gα subunits) vary in length from 41 amino acids (GPA1) to 31 amino acids (Gαt). Most Gα subunits share the consensus sequence for the addition of myristic acid at their amino termini (MGXaaS-) (SEQ ID NO:82), although not all Gα subunits that contain this motif have myristic acid covalently associated with the glycine at position 2 (Speigel, *et al.* (1991) TIBS 16:338-3441). The role of this post-translational modification has been inferred from studies in which the activity of mutant Gα subunits from which the consensus sequence for myristoylation has been added or deleted has been assayed (Mumby *et al.* (1990) Proc. Natl. Acad. Sci. USA 87: 728-732; (Linder, *et al.* (1991) J. Biol Chem. 266:4654-4659); Gallego, *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:9695-9699). These studies suggest two roles for N-terminal myristoylation. First, the presence of amino-terminal myristic acid has in some cases been shown to be required for association of Gα subunits with the membrane, and second, this modification has been demonstrated to play a role in modulating the association of Gα subunits with Gβγ complexes. The role of myristoylation of the GPA1 gene products, at present is unknown.

In other biochemical studies aimed at examining the role of the amino-terminus of Gα in driving the association between Gα and Gβγ subunits, proteolytically or genetically truncated versions of Gα subunits were assayed for their ability to associate with Gβγ complexes, bind guanine nucleotides and/or to activate effector molecules. In all cases, Gα subunits with truncated amino termini were deficient in all three functions (Graf, *et al.* (1992) J. Biol. Chem. 267:24307-24314); (Journot, *et al.* (1990) J. Biol. Chem. 265:9009-9015); and (Neer, *et al.* (1988) J. Biol. Chem 263:8996-9000). Slepak, *et al.* (1993, J. Biol. Chem. 268:1414-1423) reported a mutational analysis of the N-terminal 56 amino acids of mammalian Gαo expressed in Escherichia coli. Molecules with an apparent reduced ability to interact with exogenously added mammalian Gβγ were identified in the mutant library. As the authors pointed out, however, the assay used to screen the mutants the extent of ADP-ribosylation of the mutant Gα by pertussis toxin was not a completely satisfactory probe of interactions between Gα and Gβγ. Mutations identified as inhibiting the

interaction of the subunits, using this assay, may still permit the complexing of Gα and Gβγ while sterically hindering the ribosylation of Gα by toxin. Other work has revealed specific amino acid residues of GPA1 that are important in GPA1 function. For example, a E307K mutation appears to create an a subunit with a broadened specificity for Gβ subunits (Whiteway *et al.* 1994. Mol. Cell. Biol. 14:3223). Interestingly, the residue in the mammalian G α subunit which is equivalent to the E307 position is diagnostic for a particular class of mammalian α subunits. For example, the $G_S\alpha$ subunits contain a lysine at this position, the G_O and G_i α subunits contain a histidine, the transducin α subunits have a glutamine, the Gq α subunits have a proline, and the G_{13} a subunits have an aspartic acid at this site (Whiteway *et al.* supra).

Genetic studies examined the role of amino-terminal determinants of Gα in heterotrimer subunit association have been carried out in both yeast systems using GPA1-mammalian Gα hybrids (Kang, *et al.* (1990) Mol. Cell. Biol. 10:2582-2590) and in mammalian systems using Gαi/Gαs hybrids (Russell and Johnson (1993) Mol. Pharmacol. 44:255-263). In the former studies, gene fusions, composed of yeast GPA1 and mammalian Gα sequences were constructed by Kang, *et al.* (supra) and assayed for their ability to complement a gpa1 null phenotype (i.e., constitutive activation of the pheromone response pathway) in S. cerevisiae. Kang, *et al.* demonstrated that wild type mammalian Gαs, Gαi but <u>not</u> Gαo proteins are competent to associate with yeast Gα and suppress the gpa1 null phenotype, but only when overexpressed. Fusion proteins containing the amino-terminal 330 residues of GPA1 sequence linked to 160, 143, or 142 residues of the mammalian Gαs, Gαi and Gαo carboxyl-terminal regions, respectively, also coupled to the yeast mating response pathway when overexpressed on high copy plasmids with strong inducible (CUP) or constitutive (PGK) promoters. All three of these hybrid molecules were able to complement the gpa1 null mutation in a growth arrest assay, and were additionally able to inhibit α–factor responsiveness and mating in tester strains. These last two observations argue that hybrid yeast-mammalian Gα subunits are capable of interacting directly with yeast Gβγ, thereby disrupting the normal function of the yeast heterotrimer. Fusions containing the amino terminal domain of Gαs, Gαi or Gαo, however, did not complement the gpa1 null phenotype, indicating a requirement for determinants in the amino terminal 330 amino acid residues of GPA1 for association and sequestration of yeast Gβγ complexes. Taken together, these data suggest that determinants in the amino terminal region of Gα subunits determine not only the ability to associate with Gβγ subunits in general, but also with specific Gβγ subunits in a species-restricted manner.

specific peptide to interact with the receptor during transit through the secretory pathway. This has been postulated to occur in mammalian cells exhibiting autocrine activation. Such interaction could yield activation of the response pathway during transit, which would still allow identification of those cells expressing a peptide agonist. For situations in which peptide antagonists to externally applied receptor agonist are sought, this system would still be effective, since both the peptide antagonist and receptor would be delivered to the outside of the cell in concert. Thus, those cells producing an antagonist would be selectable, since the peptide antagonist would be properly and timely situated to prevent the receptor from being stimulated by the externally applied agonist.

An alternative mechanism for delivering peptides to the periplasmic space is to use the ATP-dependent transporters of the STE6/MDR1 class. This transport pathway and the signals that direct a protein or peptide to this pathway are not as well characterized as is the endoplasmic reticulum-based secretory pathway. Nonetheless, these transporters apparently can efficiently export certain peptides directly across the plasma membrane, without the peptides having to transit the ER/Golgi pathway. It is anticipated that at least a subset of peptides can be secreted through this pathway by expressing the library in context of the α-factor prosequence and terminal tetrapeptide. The possible advantage of this system is that the receptor and peptide do not come into contact until both are delivered to the external surface of the cell. Thus, this system strictly mimics the situation of an agonist or antagonist that is normally delivered from outside the cell. Use of either of the described pathways is within the scope of the invention.

The present invention does not require periplasmic secretion of peptides, or, if such secretion is provided, any particular secretion signal or transport pathway. In certain embodiments, peptides expressed with a signal sequence may bind to and activate receptors prior to their transport to the cell surface.

B. GPCR Expression

In other embodiments, a leader sequence of a yeast secreted protein can be used to direct transport of receptors, for example, G-protein coupled receptors to the plasma membrane as described in detail in the appended examples. Previous work has demonstrated the expression of foreign, secreted proteins in yeast cells using the α-factor leader. However, when a heterologous membrane bound receptor, the rat M5 receptor, was expressed using such a system, it was found that the heterologous GPCR did not functionally integrate into the yeast cell signaling pathway (Huang *et al.*

residues from a heterologous G α subunit. In another embodiment, the last 6 C-terminal amino acid residues, i.e. positions 467-472 of Gpa1, can be removed and replaced with C-terminal amino acid residues from a heterologous G α subunit. In another embodiment, the last 28 amino acid residues, i.e. positions 445-472 of Gpa1, can be removed and replaced with C-terminal amino acid residues from a heterologous G α subunit. In another embodiment, the last 30 amino acid residues, i.e. positions 443-472 of Gpa1, can be removed and replaced with C-terminal amino acid residues from a heterologous G α subunit. In yet another embodiment, the last 50 amino acid residues, i.e. positions 423-472 of Gpa1, can be removed and replaced with C-terminal amino acid residues from a heterologous G α subunit. In various other embodiments, any number of C-terminal Gpa1 amino acid residues from 4 to 50 amino acids can be replaced, e.g., 4-50, 4-40, 4-30, 4-28, 4-6, more preferably, 5 or 6.

The C-terminal amino acids of the Gpa1 are replaced with the C-terminal amino acids from a heterologous G α subunit. For example, the last 5 C-terminal amino acids of Gpa1, i.e., positions 468-472, are removed and replaced with the last 5 C-terminal amino acids of a heterologous G α subunit. The C-terminal amino acids of the Gpa1 amino acids can be replaced with C-terminal amino acids of essentially any heterologous G protein α subunit, but most preferably are replaced with mammalian sequences, most preferably mouse, rat or human sequences. The heterologous G α subunits can be selected from any member of the G α subunit family, for example Gαs, Gαi2, Gαi3, Gαq, $G\alpha o_a$, $G\alpha o_b$ and Gα16. Preferably, the G α subunit is selected from Gαs, Gαi2, Gαi3, Gαq, and Gα16.

In one embodiment, from the last 4 to the last 50 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In another embodiment, from the last 4 to the last 40 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In yet another embodiment, from the last 4 to the last 28 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In a preferred embodiment, from the last 4 to the last 6 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In a more preferred embodiment, the last 5 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In various other embodiments, any number of C-terminal amino acid residues of the heterologous G α subunit from 4 to 50 amino acids can replace Gpa1 sequences, e.g., 4-50, 4-30, 4-40, 4-28, 4-6, more preferably, 5 or 6.

In one embodiment, from the last 4 to the last 50 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In another embodiment, from the last 4 to the last 40 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In another embodiment, from the last 4 to the last 28 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In a preferred embodiment, from the last 4 to the last 6 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In a more preferred embodiment, the last 5 C-terminal amino acids of the heterologous G α subunit replace the C-terminal amino acids of Gpa1. In various other embodiments, any number of C-terminal amino acid residues of the heterologous G α subunit from 4 to 50 amino acids can replace Gpa1 sequences, e.g., 4-50, 4-40, 4-30, 4-28, 4-6, more preferably, 5 or 6.

Additionally, for sandwich chimera, N-terminal amino acids of a heterologous G α subunit replace or are added onto N-terminal amino acids of Gpa1. Thus, the N-terminus of the Gpa1 is "operably linked" to the N-terminal amino acids of a heterologous G α subunit. The N-terminal amino acids can be derived from the same heterologous G α subunit as the C-terminal sequences or from a different heterologous G α subunit. Most preferably, the heterologous N-terminal amino acids are from a mammalian G α subunit, most preferably mouse, rat or human sequences. The heterologous G α subunits can be selected from any member of the G α subunit family, for example Gαs, Gαi2, Gαi3, Gαq, $G\alpha o_a$, $G\alpha o_b$ and Gα16. Preferably, the G α subunit is selected from Gαs, Gαi2, Gαi3, Gαq, and Gα16.

The N-terminal amino acids of Gpa1 can be deleted and replaced with N-terminal amino acids of a heterologous (e.g. mammalian) G α subunit, for example, N-terminal amino acids 1-50 of Gpa1, can be removed and replaced with N-terminal amino acids from the heterologous G α subunit. In another embodiment, N-terminal amino acids 1-30 of Gpa1 can be removed and replaced with N-terminal amino acids from the heterologous G α subunit. In a preferred embodiment, N-terminal amino acids 1-21 of the Gpa1 are removed and replaced with N-terminal amino acids from the heterologous G α subunit. In another preferred embodiment, N-terminal amino acids 1-11 of Gpa1 are removed and replaced with N-terminal amino acids from the heterologous G α subunit.

In one embodiment, the first 50 N-terminal amino acids of the heterologous G α subunit replace or are added onto the N-terminal amino acids of Gpa1. In another embodiment, the first 40 N-terminal amino acids of the heterologous G α subunit

replace or are added onto the N-terminal amino acids of Gpa1. In another embodiment, the first 30 N-terminal amino acids of the heterologous G α protein subunit replace or are added onto the N-terminal amino acids of Gpa1. In another embodiment, the first 21 N-terminal amino acids of the heterologous G α protein subunit replace or are added onto the N-terminal amino acids of Gpa1. In yet another embodiment, the first 11 N-terminal amino acids of the heterologous G α protein subunit replace or are added onto the N-terminal amino acids of Gpa1. In various other embodiments, any number of N-terminal amino acid residues of the heterologous G α subunit from 5 to 50 amino acids can replace or be added onto N-terminal Gpa1 sequences, e.g., 5-50, 5-40, 5-30, 5-21 or 5-11.

In one embodiment, replacements to the C-terminal and N-terminal amino acids of Gpa1 can be made using the same heterologous G α subunit. In one embodiment, this heterologous G α subunit is the Gαq. The skilled artisan will appreciate that replacements can be made with any member of the G α subunit family. Also within the scope of the invention, the replacements at the N- and C-termini can be made with different G α subunits at the C-terminal and N-termini of Gpa1. Examples of combinations of C-terminal and N-terminal replacements include, but are not limited to, Gαs-Gpa1-Gαq; Gαq-Gpa1-Gαs; Gαs-Gpa1-Gαi2; Gαi2-Gpa1-Gαs; Gαs-Gpa1-Gαi3; Gαi3 -Gpa1-Gαs, and the like.

The Gpa1 sequence preferably is replaced with a wild type sequence of a heterologous G α subunit, for example by replacing the last 5 C-terminal amino acids of the Gpa1 with the corresponding last 5 C-terminal amino acids of a wildtype mammalian G α subunit. However, the Gpa1 sequence may also be replaced with sequences of a mutant G α subunit containing, for example, one or more conservative substitutions in the last 5 C-terminal amino acid sequence of the G α subunit. Mutant G α subunit sequences are selected based on their ability to retain, or improve, receptor specificity. Accordingly, the C-terminal amino acids of the heterologous Gα subunit are intended to include wildtype sequences and conservative substitutions that maintain receptor specificity.

Coupling specificity of the chimeric Gα proteins can be tested by expressing the sandwich chimeric G proteins in yeast cells that also express a heterologous G protein-coupled receptor. Coupling of the sandwich chimeric G proteins to a heterologous receptors can be determined by, for example, Fus1-pHIS3 screening, as described in the Example 12. Sandwich chimeric G proteins in which C-terminal and N-terminal amino acids of Gpa1 are replaced by sequences of heterologous G α subunits demonstrate an

same enzymes, to yield Cp3635, encoding a Gpal(1-43)-Gαi2(36-242)-GαoB(243-354) chimera containing the above changes.

Plasmids encoding GPA1-Gα12 chimerae were constructed as follows. The single Pst I site in Cp1127 was eliminated by digesting with BamH1 and Pst1, blunting the overhangs with T4 DNA polymerase, and ligating the resulting linear DNA; the resulting plasmid (Cp3326) was digested with NcoI and SacI, allowing insertion of a synthetic oligonucleotide encoding the N-terminus 41 amino acids of GPA1p. The synthetic oligonucleotide, which contains a Pst I site at condons 18 and 19 of GAP1, was made by annealing phosphorylated oligonucleotide o207 (AAAAGAGCCAATGATGTCATCGAGCAATCGTTGCAGCTGGAGAAACAACGTGACAAGA ATGAGCT) (SEQ ID NO:67) with oligonucleotide o208 (CATTCTTGTCACGTTGTTTCTCCAGCTGCAACGATTGCTCGATGACATCATTGGCTCT TTTGTTC) (SEQ ID NO:68) and oligonucleotide o209 (CATGGGGTGTACAGTGAGTACGCAAACAATAGGAGATGAAAGTGATCCTTTTCTGCAG AAC) (SEQ ID NO:69) with phosphorylated oligonucleotide o210 (TGCAGAAAAGGATCACTTTCATCTCCTATTGTTTGCGTACTCACTGTACACCC) (SEQ ID NO:70), followed by their ligation. The plasmid resulting from the insertion of this approximately 120 bp synthetic DNA fragment, Cp3363, was then digested with SacI and XhoI, permitting the insertion of a PCR-amplified, SacI- and XhoI-digested fragment encoding amino acids 43 through the stop codon of wild-type Gα12. The resulting plasmid Cp3435 thus encodes a chimeric GPA1-Gα12 protein in which the N-terminal 41 residues of GPA1 replace the N-terminal 42 amino acids of wild-type Gα12. An equivalent PCR-amplified, SacI- and XhoI-digested fragment encoding amino acids 43 through the stop codon of the GTPase-deficient mutant of Gα12 was also inserted into Cp3363. The resulting plasmid Cp3436 encodes a chimeric protein in which the N-terminal 41 residues of GPAI replace the N-terminal 42 amino acids of the Q229L mutant of Gα12.

Cp3435 was used as the template for mutagenesis by Stratagene's Quik-Change protocol using oligonucleotides o286 (CTTCTTCAACGTCCCCATCATCCTC) (SEQ ID NO:71) and o287 (GAGGATGATGGGGACGTTGAAGAAG) (SEQ ID NO:72) to create Cp3822. Cp3822 encodes a GPA1-Gα12 chimera in which the serine corresponding to residue 288 of wild-type Gα12 has been changed to a proline. Cp3435 was also the template for mutating the glycine corresponding to residue 228 of wild-type Gα12 to alanine using oligonucleotides o293 (GGATGTGGGCGCCCAGAGGTCACAG) (SEQ ID NO:73) and o294

a ~10 fold increase in β-galactosidase units, demonstrating the ability of the mutant Gαs to interact productively with receptor, and to dissociate from βγ upon ligand addition.

The S286P mutation suggested a possible method for expanding the spectrum of Gα subunits that can interact with yeast Gβγ. It was reasoned that because all mammalian Gα subunits possess a serine at this position and a proline is found in Gpal, the S286P substitution might be extrapolated to other mammalian Gα subunits to improve their apparent Gβγ affinity. It has previously been shown that the substitution of the first 43 amino acids of yeast Gpa1, corresponding to the amino terminal alpha-helical domain, for the corresponding segment of Gαi2 results in a dramatic increase in the apparent affinity of Gαi2 for the yeast Gβγ subunit, as revealed by suppression of the constitutive Gβγ pheromone signal ("41-i2"). This modification to Gαl6 and Gα12 improved their ability to sequester yeast βγ, and this affinity is further strengthened by the substitution of the appropriate serine residue (corresponding to position 286 in Gαs) with proline. In addition, the apparent βγ affinity of the triple chimera Gpal(1-43)-Gα i2(36-242)-GαoB(243-354) was also significantly improved by the appropriate S → P substitution.

Example 3. Construction of STE18/ mammalian Gγ chimeric proteins

Chimeric proteins comprising STE18, the wild type Gγ subunit of yeast, were also made. The wild type STE18 nucleotide sequence is available in the art. The carboxy terminal 34 amino acids of STE18 are as follows:

GYPVAGSNHFIEGLKNAQKNSQMSNSNSVCCTLM (SEQ ID NO: 29)

The wild type human Gγ nucleotide sequences are available in the art. The carboxy terminal 24 amino acids of human Gγ are as follows:

DPLLTPVPASENPFREKKFFCAIL (SEQ ID NO:30)

The underlined residues shown are conserved among all of the mammalian Gγ subunits

The STE18-mammalian Gγ chimeras were constructed using standard molecular biology techniques. The following is a list of the Gγ chimeras which were

The following abbreviations are used in the Table. VIP (vasoactive intestinal peptide); ML1b (melatonin receptor); C5a (complement cascade component); FPRL (formyl peptide related receptor); IL-8 (interleukin 8); A2a (Adenosine 2a receptor)

Example 6. Development of four yeast strains for orphan receptor expression.

This Example illustrates the development of four yeast strains, each expressing different chimeric G protein subunits, for use in drug screening assays. The use of four different types of G protein subunits in the yeast cells provides an opportunity to achieve optimal G protein receptor coupling in at least one of the yeast strains. The genotypes of four exemplary yeast strains are illustrated in the table below:

Genotypes of Four exemplary yeast strains for Orphan Receptor Expression

CY10103	MATα ste18γ6-3841 gpal(41)-Gαi2 far1Δ1442 cyh2 tbt1-1 fus1-HIS3 can1 ste14::trp1::LTS2 ste3Δ1156 lys2 ura3 leu2 trp1 his3 ade2Δ3447 ade8Δ3457
CY10132	MATα ste18γ6-3841 gpal(41)-Gα16(S270P) far1Δ1442 tbt-1 fus1-HIS3 can1 ste14::trp1::LYS2 ste3Δ1156 lys2 ura3 leu2 trp1 his3 ade2Δ3447 ade8Δ3457
CY10150	MATα ste18γ6-3841 gpal(41)-Gαs(D229S) far1Δ1442 tbt1-1 fus1-HIS3 can1 ste14::trp1::LYS2 ste3Δ1156 lys2 ura3 leu2 trp1 his3 ade2Δ3447 ade8Δ3457
CY10560	MATα ste18γ6-3841 far1Δ1442 tbt1-1 fus1-HIS3 can1 ste14::trp1::LYS2 sst2 Δste3Δ1156 lys2 ura3 leu2 trp1 his3 ade2Δ3447 ade8Δ3457

As discussed above, Gα and Gγ chimeras are integrated at the gpa1 and ste18 loci respectively. Fus1-HIS3 is integrated at the fus1 locus and is phenotypically fus1 minus. A listing of phenotypes associated with the genotypes listed above is provided below.

MATα	mating type resulting in production of α-factor and responsiveness to **a**-factor
ste18γ6	chimeric yeast Gγ/Human Gγ2, enchances interaction with receptor
gpal(41)-Gαi2	chimeric yeast Gα/Human Gαi2, enchances interaction with receptor
gpal(41)-Gα16(S270P)	chimeric yeast Gα/mutant Human Gα16, enchances interaction with yeast βγ
gpalp-Gαs(D229S)	mutant Human Gαs, enchances interaction with yeast βγ
far1Δ1442	eliminates growth arrest response in pheromone pathway
cyh2	recessive resistance to cycloheximide

tbt1-1	poorly characterized enhanced transformation by electroporation
fus1-HIS3	pheromone responsive histidine prototrophy (aminotriazole resistance)
can1	recessive resistance to canavinine
ste14::trp1::LYS2	eliminates carboxymethylation of isoprenylated proteins leading to reduced background through the pheromone response pathway
sst2Δ2	supersensitivity resulting from GAP activity on GPA1
ste3Δ1156	deletion of **a**-factor receptor gene
lys2	lysine auxotrophy and resistance to α-aminoadipate
ura3	uracil auxotrophy, complementation by ligand plasmids
leu2	leucine auxotrophy, complementation by receptor plasmids
trp1	tryptophan auxotrophy, complementation by fus1-lacZ plasmid
his3	histidine auxotrophy, complementation by fus1-HIS3
ade2Δ3447	adenine auxotrophy, leads to generation of red pigment
ade8Δ3457	adenine auxotrophy, eliminates generation of red pigment in ade2 cells, complemented by receptor plasmid leading to colorimetric verification of its presence

Example 7: Construction of the α-Factor Leader-based Expression Vectors

A yeast vector for the expression of mammalian G protein-coupled receptors fused to a leader sequence of prepro-α-factor has been constructed as follows. A 0.38-kb fragment including a transcription terminator of yeast gene PH05 was amplified by PCR using a plasmid pTER as a template. The latter plasmid was constructed by subcloning of a Sau3A-PstI fragment of PH05 gene (GenBank accession number A07173) into the vector pUC19 digested with BamHI and PstI. PCR primers used were TER1, 5'-GGATCTAGAGGATCCTGGTACGTTCCTC-3' (SEQ ID NO: 6) , and TER2, GTCGCTAGCCAAGCTTGCATGCCTGCAG-3' (SEQ ID NO: 7) (BRL, Life Technologies, Gaithersburg, MD). The primers provided XbaI and NheI restriction sites at the 5'-and 3'-terminus of the amplified fragment, respectively. The total of 30 cycles of PCR was performed; each cycle included denaturation at 94°C for 45 sec, annealing at 53°C for 1 min, and polymerization at 72°C for 1.5 min. The amplified fragment was digested with XbaI and NheI and subcloned into XbaI site of the plasmid Cadus 1289 ($pLPX_t$) (LEU2 PGKp 2mu-ori REP3 AmpR) in appropriate orientation.

There was however, an alternative interpretation. Large, highly glycosylated proteins transfer to nitrocellulose with low efficiency. Therefore, it was a formal possibility that receptor lacking a yeast leader was hyper-glycosylated, rendering it resistant to detection by Western blot. To address this possibility, protein samples were treated with Endoglycosidase H (Endo H), which cleaves the high mannose structures on N-linked oligosaccharides. Removal of oligosasccharides did not improve visualization of the leader receptor, suggesting that in the vector, the mGlu2R was not synthesized. Furthermore, the Endo H results indicated the presence of oligosaccharides on receptor made form constructs with leaders, once again indicating transport into the secretory pathway. Together, these data underscore the important role yeast leader sequences play in mGlu2 receptor expression.

Several agonists are available for research on the mGlu2 receptor. The agonists used in these studies included L-CCG-I, 1S,3R-ACPD, and L-glutamic acid, with EC_{50} values of 0.75 μM, 7.7 μM and 11.8 μM, respectively. Stimulation of the mGlu2 receptor has been demonstrated to promote inhibition of adenylyl cyclase, thus initial attempts to couple the receptor focused primarily upon the use of proteins from the $G_{\alpha i}$ family.

CY9437 expresses a unique Ste18-Gγ2 chimera. To date, most receptors that have been developed into successful assays have shown the ligand independent receptor activation, LIRMA, phenotype in this strain when presented with an appropriate G_{α} subunit. Therefore in the absence of receptor coupling, CY9437 can be loosely used as a diagnostic indicator of the capacity of a receptor to couple in yeast. When compared with other mGlu2 receptor constructs, only ppαF-mGluR2Δ induced LIRMA. However, it is possible that rather than LIRMA, this is actually ligand-dependent activation arising from glutamate released into the growth media by the yeast. Glutamate oxidase, which converts glutamate to α-ketoglutarate, was used in an attempt to diminish glutamate levels. Nonetheless, additional glutamate metabolizing enzymes (which may be more potent under yeast growth conditions) are available including L-glutamate decarboxylase and L-glutamate dehydrogenase.

Example 11. Construction of Gpa1 carboxy terminal chimera.

The C-terminus of G protein α subunits have been shown to be crucial in determining receptor-G protein specificity (see e.g., Conklin *et al.*, (1995) Nature 363, 274-276; Liu *et al.*, (1995) Proc. Natl. Acad. Sci. 92, 111642-116460; Hamm *et al.*, (1998) J. Biol. Chem. 273:669-672; Conklin *et al.*, (1996) Mol. Pharmacol. 50:885-890). To test the ability for specific coupling through heterologous receptors expressed in yeast,

GPA1-Gαo(5) Primer 1: 5'TTT GAA GGG CCG TAT AAA GAC3' (SEQ ID NO: 87)
Primer 10: 5'ACG TCT CGA GAT CGA CTC AAT ATA GAC CAC ATC CTT TAA GGT TTT GCT GG3' (SEQ ID NO: 96)

Following amplification, the PCR products, which were approximately 550bp in size, were column purified using the Qiagen PCR purification kit. The purified amplification product was digested with BstBI and XhoI for cloning into Cadus 1179. Cadus 1179 was digested with BstBI and XhoI, treated with shrimp alkaline phosphatase and purified by gel electrophoresis. The PCR product and linearized vector were ligated and electroporated into competent bacteria. Colonies grown on ampicillin plates were selected and amplified. The DNA was isolated from the bacterial cells and sequenced to provide the following C-terminal chimera:

Alignment of GPA1 and mammalian Gα subunits:

Gpa1: .....AVTDLIIQQNLK**KIGII** (SEQ ID NO: 97)
ai2: .....AVTDVIIKNNLK**DCGLF** (SEQ ID NO: 98) (ai1 has the same last 5aa)
aq: .....AVKDTILQLNLK**EYNLV** (SEQ ID NO: 99)(a11 has the same last 5aa)
a12: .....AVKDTILQENLK**DIMLQ** (SEQ ID NO: 100)
as: .....DCRDIIQRMHLR**QYELL** (SEQ ID NO: 101)
a13: .....DVKDTILHDNLK**QLMLQ** (SEQ ID NO: 102)
az: .....AVTDVIIQNNLK**YIGLC** (SEQ ID NO: 103)
a16: .....DVRDSVLARYL**DEINLL** (SEQ ID NO: 104)
as: .....DCRDIIQRMHL**RQYELL** (SEQ ID NO: 105)
ao: .....AVTDIIIANNLR**GCGLY** (SEQ ID NO: 106)

The C-terminal amino acids of S. cerevisiae GPA1 and mammalian Gα subunits are shown above. For each mammalian Gα subunit shown, the amino acids which were exchanged with amino acids at the C-terminus of GPA1 are shown in bold and underlined.

The last 5 amino acids of GPA1 were substituted with the last 5 amino acids from the corresponding mammalian Gα subunit to create the following GPA1-Gα(5) chimeras:

GPA1-Gαi2(5) (SEQ ID NO: 107)
GPA1-Gαq(5),(SEQ ID NO: 108)

GPA1-Gα12(5),(SEQ ID NO: 109)
GPA1-Gαs(5),(SEQ ID NO: 110)
GPA1-Gα13(5),(SEQ ID NO: 111)
GPA1-Gαz(5),(SEQ ID NO: 112)
GPA1-Gαo(5) (SEQ ID NO: 113)

The last 6 amino acids of GPA1 were substituted with the last 6 amino acids from the corresponding mammalian Gα subunit to create the following GPA1-Gα(6) chimeras:
GPA1-Gα16(6)(SEQ ID NO: 114)
GPA1-Gαs(6)(SEQ ID NO: 115)

A summary of chimeric G proteins is presented in Table 1. Sandwich chimeric G proteins are also shown in Table 1 and will be discussed in more detail in Example 12.

Table 1 Gpa1 -Gα(5), Gpa1-Gα (6) and sandwich chimeras

G protein	**Cadus Plasmid Number**
GPA1-Gαi2(5)	Cp4921, 4920
GPA1-Gαs(5)	Cp5007, 5008
GPA1-Gα12(5)	Cp5040, 5041
GPA1-Gαq(5)	Cp5042, 5043
GPA1-Gαz(5)	Cp5169, 5170
GPA1-Gα13(5)	Cp5407, 5408
GPA1-Gα16(6)	Cp5531, 5532
GPA1-Gαs(6)	Cp5905
Gαq(1-11)-GPA1(6-467)-Gαq(355-359)	Cp5902
Gαq(1-21)-GPA1(24-467)-Gαq(355-359)	Cp6079
GPA1-Gαo(5)	Cp6193

Construction of Yeast Strains with Integrated G-proteins:

GPA1 chimerae were integrated into the yeast genome such that the native GPA1 open reading frame was replaced with the chimeric open reading frame.

CADUS 1584 + CADUS 2311 LacZ$^+$ FPRL1 Receptor$^+$
CADUS 1584 + CADUS 2695 LacZ$^+$ Melatonin 1a Receptor$^+$
CADUS 1584 + CADUS 1289 LacZ$^+$ Receptor$^+$

A pool of transformants was picked from each transformation and grown overnight in media lacking tryptophan and leucine, at pH6.8 with 25mM PIPES. The optical density at 600 nm of a 1/10 dilution of the overnight cultures was determined and the cultures were diluted in fresh media to final OD_{600} of 0.2. Strains with the melatonin receptor were then grown for an additional 1.5 hours and diluted again to an OD_{600} of 0.2. The LacZ enzyme activity was determined at increasing concentrations of ligand. The LacZ enzyme assay was performed in a 96 well plate and each reaction was performed in triplicate in a total volume of 100 µl. For each reaction, 90 µl of culture and 10 µl of ligand were used. The final concentration of DMSO in each well was kept constant at 1% for the FPRL1 receptor and at 5% for the Melatonin la receptor.

LacZ activity, in the presence and absence of the FPRL1 receptor, was measured at the following concentrations of FPRL1: 0nM, 1.6nM, 8nM, 40nM, 200nM, 1µm, and 25µM. Following the addition of the ligand, the 96 well plates were incubated at 30C for 4 hours. 20µl of 0.5mM fluorescein di-β-D-galactopyranoside (FDG) was added as a substrate of the reaction. The plates were incubated at 37C for 90 mins. Following incubation, the reaction was stopped by the addition of 20ml 1M Na_2CO_3 to each well. Plates were read using a fluorometer at an excitation wavelength of 485nm and an emission wavelength of 535nm.

LacZ activity, in the presence and absence of the melatonin receptor, was measured at the following concentration of melatonin: 0pM, 1pM, 10pM, 100pM, 1nM, 10nM, 100nM, 1µM, 10µM, 100µM, 1mM and 4.3mM. Results show that the Gpa1, αi2 subunit and the C-terminal αi2 (5) protein chimera couple and stimulate the FPRL1 receptor. With the melatonin receptor, stimulation of the receptor was observed with the chimeric G protein, as with wild type Gpa1. Coupling was also observed with the Gpa1-41αi2 chimeric protein, although the stimulation of the receptor with this chimeric protein was less efficient. These results demonstrate that chimeric G proteins are able to couple and stimulate some heterologous receptors.

Stimulation through various other heterologous receptors was investigated using the chimeric G proteins. A summary of the results is presented in Table 3. Data from Table 3 shows that stimulation of heterologous receptors is observed with several chimeric G proteins in which Gpa1 alone failed to stimulate.

Receptor	GPA1 and GPA1 chimeras	
	functional	**nonfunctional**
CXCR2 (mutant)	GPA1 GPA1-Gαi2(5) GPA1-Gα16(6)	
CXCR4	GPA1-Gαi2(5)	GPA1
Adenosine 2b receptor	GPA1-Gαs(5)	GPA1
Bombesin receptor subtype 3	GPA1-Gαz(5) Gαq(1-11)-GPA1(6-467)-Gαq(355-359)(FUS1p-HIS3)	GPA1 GPA1-Gαq(5)
Bradykinin receptor 2	GPA1-Gαq(5) Gαq(1-11)-GPA1(6-467)-Gαq(355-359)	GPA1

Coupling to heterologous orphan receptors using C-terminal chimeric G proteins or sandwich chimeras was tested using the lacZ assay. The results of the assay are shown in Table 5. Data demonstrates that stimulation via orphan receptors was observed using the chimeric G proteins (referred to as "tail' in Table 5). Stimulation was also observed with at the Gαq(1-11)-GPA1(6-467)-Gαq(355-359) sandwich chimera.

Table 5 Table showing orphan receptors and C-terminal chimeric G proteins or sandwich chimeras

Receptor	**GPA1**	**i2 tail**	**12 tail**	**q tail**	**s tail**	**16 tail**	**13 tail**	**Q sand-wich (1-11)**	**S(6) tail**	**Q sand-wich (1-21)**	**z tail**
Orphan 1	X	-	-	-	X*	-	-	-	-	-	-
Orphan 2	X	X	-	-	-	ND	-	ND	ND	ND	-
Orphan 3	X	X	X	-	-	ND	ND	ND	ND	ND	ND
Orphan 4	X	-	X*	-	-	ND	ND	ND	ND	ND	ND
Orphan 5	X	X	-	-	X	ND	-	ND	ND	ND	X
Orphan 6	-	-	-	X	X*	-	-	ND	ND	ND	-
Orphan 7	X	X	-	X	-	ND	ND	ND	ND	ND	ND
Orphan 8	X	-	-	-	-	-	-	ND	ND	ND	-
Orphan 9	-	X*	X*	-	-	-	X*	-	-	-	X*
Orphan 10	-	X*	X*	X*	-	ND	X*	ND	ND	ND	X*

Orphan 11	X	-	-	weak	weak	ND	-	X	-	-	-
Orphan 12	X	weak	weak	X*	-	ND	ND	ND	ND	ND	ND
Orphan 13	X	-	X	-	-	ND	-	ND	ND	ND	-
Orphan 14	X	X	-	weak	-	ND	X	ND	ND	ND	X
Orphan 15	-	-	X	-	-	-	-	weak	X	ND	-
Orphan 16	-	-	-	X*	-	-	-	X	ND	ND	-
Orphan 17	X	X	X	X	X	X	-	ND	ND	ND	X

X works

X* works better in tails. ND: not done

Example 12. Construction of "sandwich" chimera G proteins.

In addition to the C-terminus of G protein α subunits being crucial in determining receptor-G protein specificity, the N-terminus of G protein a subunit also appears to be involved in receptor contact (see e.g., Hamm *et al.*, (1998) J. Biol. Chem. 273:669-672). Sandwich chimeric proteins were constructed to investigate the effect on receptor specificity by substituting both the C-terminal and N-terminal regions of the Gpa1 with protein G α subunits.

Construction of Gαq(1-11)-GPA1(6-467)-Gαq(355-359)

The Gαq(1-11)-GPA1(6-467)-Gαq(355-359) sandwich chimera was constructed in which the first 11 amino acids of Gαq and residues 6-381 of GPA1 was constructed using Cp1179 as a template. The following primers were used for PCR amplification:

Primer11:ACGTGGTCTCCCATGACTTTGGAATCTATTATGGCTTGTTGTCTTAG TACGCAAACAATAGGAGACG (SEQ ID NO: 116)

Primer12: GTATCTTTGAACCACTTAGAG (SEQ ID NO: 117)

The PCR product containing the first 11 amino acids of Gαq and residues 6-381 of GPA1, was purified and digested with BstB1 and BsaI. The digested product was ligated into Cp5042 (GPA1p-GPA1-Gαq(5) CEN6 ARS4 AmpR TRP1). The ligation mixture was electroporated into competent bacteria. Plasmid DNA was prepared from ampicillin resistant bacterial colonies and the sequence of the G protein was verified by DNA sequence analysis. One clone which contained the 11 amino acids of Gαq was identified and referred to as Cp5902: GPA1p-Gαq(1-11)-GPA1(6-467)-Gαq(355-359) CEN6 ARS4 AmpR TRP1, (SEQ ID NO: 118).

Construction of Gαq(1-21)-GPA1(24-467)-Gαq(355-359)

The Gαq(1-21)-GPA1(24-467)-Gαq(355-359) sandwich chimera was constructed in which residues 13-21 of Gαq and residues 23-381 of GPA1 was constructed using Cp5902 as a template. PCR amplification was performed using two sets of primers. The first set of primers had the following sequence:
Primer13: GTCTAAAATGAAGAGGATAGTAG (SEQ ID NO: 119)
Primer14: GATCCGTCTCACTTCAGAAAGACAACAAGCCATAATAG (SEQ ID NO: 120)

The first set of primers was used to generate a PCR product which contained the sequences of the GPA1 promoter downstream from the EcoRI site and the first 12 amino acids of Gαq. The PCR product was purified and digested with EcoRI and BsmBI.

A second set of primers was used to amplify residues 13-21 of Gαq and residues 23-381 of GPA1 using Cp5902 as a template and the following primers sequences:
Primer15:GATCCGTCTCTGAAGAAGCTAAGGAGGCTAGAAGAATTAATGATG TCATCGAGCAATCGTTGC (SEQ ID NO: 121)
Primer12: GTATCTTTGAACCACTTAGAG (SEQ ID NO: 122)

The resulting PCR product containing residues 13-21 of Gαq and residues 23-381 of GPA1, was purified and digested with BsmBI and BstBI.

The above two digested PCR products were then ligated into Cp5042 (GPA1p-GPA1-Gαq(5) CEN6 ARS4 AmpR TRP1), which was digested with EcoRI and BstBI. The ligation mixture was electroporated into competent bacteria. Plasmid DNA was prepared from ampicillin resistant bacteria and the sequence of the G protein was verified by DNA sequence analysis. One clone which contained the 21 amino acids of Gαq was identified and referred to as Cp6079: GPA1p-Gαq(1-21)-GPA1(24-467)-Gαq(355-359) CEN6 ARS4 AmpR TRP1 (SEQ ID NO: 123). A summary of the GPA1-Gα(5), GPA1-Gα(6) and Sandwich Chimeras constructs is provided is Table 1, in Example 11. Sandwich chimera G proteins integrated into yeast cells are shown in Table 2 in Example 11. Tables 4 and 5 in Example 11, show that sandwich chimeras are able to couple to and stimulate heterologous receptors, determined using the Fus1-pHIS3 assay.

GPA1-Gq sandwich improves functional activity of a bradykinin-responsive receptor:

Construction of yeast strains with integrated G-proteins is described in Example 11. Sandwich chimera were tested for stimulation via heterologous receptors

by transforming CY2120 with human bradykinin receptor 2 under the control of the PGK promoter, FUS1p-lacZ reporter, and one of the following plasmids: Cp1179 (GPA1), Cp5042 (GPA1-Gαq(5)), or Cp5902 (Gαq(1-11)-GPA1(6-467)-Gαq(355-359)). Transformants were grown to mid-exponential phase in medium lacking leucine, uracil, and tryptophan, at pH 6.8. 100 µl aliquots of cultures were incubated in 96-well plates in the presence of increasing concentrations of bradykinin at 30°C for 4 hours. 20 µl of 6X Z-buffer (0.6 M sodium phosphate, pH 7.0, 60 mM KCl, 6 mM $MgCl_2$, 1.6% (v/v) β-mercaptoethanol) containing 10 mM chlorophenolred-β-D-galactoside (CPRG) and 2.5% Triton X-100 was added to each aliquot followed by incubation at room temperature for 60 min. The enzymatic reactions were stopped by adding 60 µl of 1M sodium carbonate and A_{575} values were determined using a Biomek Plate Reader (Beckman). Upon ligand treatment β-galactosidase activities were not elevated for transformants with Cp1179. β-galactosidase activities were increased 26 fold for transformants with Gαq(1-11)-GPA1(6-467)-Gαq(355-359); 13 fold for transformants with GPA1-Gαq(5).

Example 13. Detection using Fus1p-GFP

To provide an rapid, efficient method of detection, GFP was used as a reporter gene. Construction of plasmids:

Constructs containing the GFP reporter gene were made by excising the lacZ gene from CP2615 (nFus1-lacz AmpR TRP1 2mu) with SalI and EagI and replacing it with the wild type GFP gene (Clontech) to produce Cp2759 (nFus1-GFPwt 2mu AmpR TRPI).

Cp2759 (nFus1-GFPwt 2mu AmpR TRPI) was used as a template to introduce mutations into the wildtype gene (Cp2759) using the quick-change mutagenesis protocol (Stratagene). The resulting plasmid, Cp4567 (nFus1-GFP F100S M154T V164A 2mu AmpR TRP1) contains a threonine at position 154 which was changed to a yeast codon biased threonine. The mutations are the same as those described by Crameri *et al.*, Nature Biotech 1996 14:315-319.

Cp5681 (nfus1p-GFPS65T 2mu AmpR TRP1) was made by digesting the Cp5600 with sites internal to GFP gene using NcoI and BstBI. The 450 base pair fragment was ligated into Cp4567 digested with the same enzymes. The Cp5681 was digested with Bg1II and EagI and the 1.1 kb fragment containing nFus1p-GFPS65T was subcloned into the multiple cloning site of following yeast integrative vectors; Cp1007 (HIS3 AmpR); Cp1009 (TRP1 AmpR); Cp1010 (LEU2 AmpR) and; Cp1011 (URA3 AmpR) that were digested with BamHI/EagI to generate: Cp5772 (nFus1GFPS65T

to 0.25 OD/ml and allowed to grow for an additional 2 ½ hrs. GFP fluorescence was determined with increasing concentrations of αFactor. αFactor was added to 6 mls YEPD (in triplicate) at the following concentrations: 1pM, 10pM, 100pM, 1nM, 10nM, 100nM, 1μM. Cultures were incubated at 30°C with ligand for 2hrs. Cells were harvested at 1.1-1.5 OD/ml. Yeast cells at 6.5 ODs (6.5×10^7 cells/ml) were centrifuged and washed once with sterile water. The yeast cells were resuspended in 100μl of sterile water and transferred to 96 well plate to be read by fluorometer at excitation wavelength 485nm, an emission wavelength of 535 nm and constant gain of 50.

For measurements are taken using a FACs machine and GFP.

The dose response for Ste2 was investigated using CY16363 which was grown overnight in YEPD 30°C. After overnight culture, the cells were diluted to an OD_{600} of 0.2 OD/ml. The yeast cells were grown for and additional 2 ½ hrs or until an OD_{600} 0.4-0.6 OD/ml was attained. αFactor was added to 6 mls YEPD (in triplicate) at the following concentrations: 1pM, 10pM, 100pM, 1nM, 10nM, 100nM, 1μM. Cultures were incubated at 30°C with ligand for 2hrs. The yeast cells were induced with ligand for 2 ½ hrs at 30°C until OD_{600} is about 0.8-1.0 OD/ml. Yeast cells were harvested and washed once with water. The yeast cells were resuspended in water to a final concentration OD.0.1D/ml. Typically, 10,000-50,000 cells were used for the analysis using standard FACs analysis procedures.

For measurements using the LacZ assay

For comparative purposes, the dose response for Ste2 was investigated using the lacZ assay. CY16363 was grown overnight in YEPD at 30°C. After overnight culture, the cells were diluted to an OD_{600} of 0.2 OD/ml. The yeast cells were grown for and additional 2 ½ hrs or until an OD_{600} 0.5 OD/ml was attained. The yeast cells were diluted to an 0.1 OD/ml and 100μl of the cells were transferred to a 96 well plate. 1μl of αFactor was added to each well to produce final concentrations as follows: 0pM, 1pM, 10pM, 100pM, 1nM, 10nM, 100nM, 1μM. Yeast cells were induced with ligand for 3 hrs at 30°C. 20μl Triton/CPRG substrate was added to each well and incubated at 30°C for 25 min. and 45 min.

Analysis of Ste2 using fus1-GFP65T by both FACs and fluorometric analysis demonstrates a significant improvement in the sensitivity compared with and fus1-Lac Z.

Dose Response for ML1a receptor measured using a fluorometer and GFP

The dose response for the ML1a receptor was investigated using CY16639 which was grown overnight in LEU minus media at 30°C. After overnight culture, the cells were diluted to an OD_{600} of 0.25 OD/ml into 6 ml LEU6.8. Cells were grown an additional 2 ½ hrs (OD = 0.4-0.5). ML1a ligand was added in increasing concentrations as follows: 0pM, 10pM, 100pM, 1nM, 10nM, 100nM, 1μM. Cells were induced with the ligand for 2 ½ hrs at 30°C. Yeast cells at a density of 6 x 10^7 cells were harvested and washed once with water. The yeast cells were resuspended in water and transferred to 96-well plate to be read by fluorometer at excitation wavelength 485nm, an emission wavelength of 535 nm and constant gain of 50.

Dose Response for ML1a receptor measured using a FACs machine and GFP

The dose response for the ML1a receptor was investigated using CY16639 which was grown overnight in LEU minus media at 30°C. After overnight culture, the cells were diluted to 0.2 OD/ml into 2ml LEU6.8. ML1a ligand was added at the same concentrations as the fluorometer assay, described above. Cells were induced with the ligand for 2 ½ hrs at 30°C (OD = 0.7-1.2). The cells were harvested and washed once with water. The cells were resuspended in sterile water to a final OD_{600} = 0.1 OD/ml and analysed using standard FACs analysis.

Dose Response for ML1a receptor measured using the LacZ assay

For comparative purposes, the dose response for ML1a receptor was investigated using the lacZ assay. CY15438 (GPA1+3907 sst2*far1*1442 tbt1-1 fus1-HIS3 can1 ste14::trp1::LYS2 ste3*1156 lys2 ura3 leu2 trp1 his3) was grown overnight at 30°C. After overnight culture, the cells were diluted to 0.2 OD/ml. Cells were grown for an additional 3 hrs to 0.4 OD/ml. The cells were then diluted to 0.1 OD/ml and 100 μl transferred to 96-well plate. 1.2μl of the ligand was added, such that the concentration of DMSO was 0.24% for each well. The ML1a receptor was added at the following concentrations: 0pM, 1 pM, 10pM, 100pM, 1nM, 10nM, 100nM, 10μM, 100μM. The cells were incubated at 30°C for 3 hrs with ligand. 20μl of Triton/CPRG was added to each well and incubated at 37°C for 1 hr. 60μl 1M Na_2CO_3 was added to stop the reaction and the absorbance was measured at OD_{57}.

Two variants of the GFP wild-type were tested and compared to nFus1-GFPwt (Cp2759). the variants GFP F100S M154T V164A (Cp4567) and GFP S65T (Cp5681) both show higher relative fluorescence compared to the wildtype GFP. GFP S65T showed the highest fluorescence. GFP reporter genes can be used to improve sensitivity which can be used to control for visualizing uniformity or variation is the

signal within a population of cells. Analysis of ML1a GPA fus1-GFP65T by both FACs and fluorometric analysis demonstrates a significant improvement in the sensitivity compared with and fus1-Lac Z.

21. The yeast cell of claim 20, wherein said second polypeptide of said second chimeric G protein subunit is from a protein selected from the group consisting of: a mammalian Gα subunit, a mammalian Gβ subunit, and a mammalian Gγ subunit.

22. The yeast cell of claim 15, wherein an endogenous yeast pheromone system receptor protein is not produced in functional form.

23. The yeast cell of claim 15, further comprising an indicator gene that produces a detectable signal upon functional coupling of the heterologous G protein coupled receptor to the G protein.

24. The yeast cell of claim 9, wherein the cell is a *Saccharomyces cerevisiae* cell.

25. The yeast cell of claim 15, wherein said heterologous G protein coupled receptor is an orphan receptor.

26. The yeast cell of claim 4, further comprising an indicator gene that produces a detectable signal upon functional coupling of the heterologous G protein coupled receptor to the G protein.

27. An assay to identify compounds capable of modulating the dissociation of Gα and Gβγ , comprising the steps of:

(i) providing a yeast cell according to claim 15 or 26,

(ii) contacting the yeast with a test compound; and

replaced with at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different; such that expression of said chimeric G protein subunit functionally integrates said heterologous GPCR into the pheromone response pathway of said yeast cell; and

wherein modulation of the signal transduction activity of said heterologous GPCR by an extracellular signal provides a detectable signal.

54. The yeast cell of claim 53, wherein said chimeric G protein subunit comprises an endogenous yeast Gpa1 subunit in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a first heterologous G protein subunit, and in which the first five N-terminal amino acids of said Gpa1 are replaced with the first 11 N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same.

55. The yeast cell of claim 54, wherein said chimeric G protein subunit comprises an endogenous yeast Gpa1 subunit in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a first heterologous G protein subunit, and in which the first 22 N-terminal amino acids of said Gpa1 are replaced with the first 21 N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same.

56. A chimeric G-protein subunit which comprises an endogenous Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a heterologous G protein subunit.

57. The chimeric G-protein subunit of claim 56, wherein the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of a heterologous G protein subunit.

58. The chimeric G-protein subunit of claim 57, wherein the last six C-terminal amino acids of said Gpa1 are replaced with the last six C-terminal amino acids of a heterologous G protein subunit.

59. A chimeric G-protein subunit which comprises an endogenous Gpa1 subunit in which at least the last four C-terminal amino acids of said Gpa1 are replaced with at least the last four C-terminal amino acids of a first heterologous G protein subunit, and in which the N-terminus of said Gpa1 is operably linked to at least the first five N-terminal amino acids of a second heterologous G protein subunit, wherein said first and second heterologous G protein subunits are the same or different.

60. The chimeric G-protein subunit of claim 59, in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of said first heterologous G-protein subunit, and in which the first five N-terminal amino acids of said Gpa1 are replaced with the first 11 N-terminal amino acids of said second heterologous G protein subunit.

61. The chimeric G-protein subunit of claim 59, in which the last five C-terminal amino acids of said Gpa1 are replaced with the last five C-terminal amino acids of said first heterologous G-protein subunit, and in which the first 22 N-terminal amino acids of said Gpa1 are replaced with the first 21 N-terminal amino acids of said second heterologous G protein subunit.

73. The yeast cell of claim 71, wherein the first 22 N-terminal amino acids of said Gpa1 are replaced with the first 21 N-terminal amino acids of said second heterologous G protein subunit.

74. The yeast cell of claim 70, 71, 72 or 73, wherein said first and second heterologous G protein subunits are the same.

75. The yeast cell of claim 74, wherein said heterologous G protein subunits are mammalian.

76. The yeast cell of claim 75, wherein said heterologous G protein subunits are Gαq subunits.

77. The yeast cell of claim 1, 2, 3, 4, 44, 47, 51 or 53, said cell further comprising a heterologous polypeptide, wherein the heterologous polypeptide is transported to a location allowing interaction with the extracellular region of the receptor expressed in the cell membrane; and wherein the heterologous polypeptide is expressed at a sufficient level such that modulation of the signal transduction activity of the receptor by the heterologous polypeptide provides a detectable signal.

78. The yeast cell of claim 45, 46, 48, 49, 50, 52 or 77, wherein said heterologous polypeptide is a ligand of said heterologous GPCR.

79. The yeast cell of claim 78, said cell further comprising a second heterologous polypeptide, said second heterologous polypeptide also being transported